Received Date : 10-Jan-2014

Revised Date : 28-Mar-2014

Accepted Date : 04-Apr-2014

Article type : Research Article

Novel 3-Substituted Ocotillol-Type Triterpenoid Derivatives as Antibacterial Candidates

Yi Bi,[ϕ,‡]Cong Ma,[ξ,‡]Hengyuan Zhang,[Ψ,‡]Zhiwen Zhou,[Ψ]Jian Yang,[ϕ]Zhenlei Zhang,[ϕ]QingguoMeng,[ϕ,*] Peter J. Lewis,[ξ,*] and JinyiXu[Ψ,*]

[ϕ]School of pharmacy, Yantai University, Yantai 264005, P. R. China. Tel: 86-535-6706022. E-mail: qinggmeng@163.com.

[ξ]Biological Sciences, School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW 2308, Australia. Tel: 612-49215701. E-mail: Peter.Lewis@newscatle.edu.au.

[Ψ]State Key Laboratory of Natural Medicines and Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, P. R. China. Tel: 86-25-83271299. E-mail: jinyixu@china.com.

‡These authors contributed equally.

Corresponding author e-mail id :beeyee_413@163.com

Plant derived triterpenoid saponins are involved in the plant defense system by targeting bacterial membranes. A series of ocotillol-type triterpenoid derivatives were synthesized starting from PPD, one of the main components of *Panax ginseng* and their antibacterial activity against several representative bacteria were evaluated. Compounds **5** and **11** exhibited excellent antibacterial activity with MIC values of 1 μg/mL against *Staphylococcus aureus* and 8 μg/mL and 4 μg/mL against *Bacillus subtilis*, respectively. Furthermore, when compounds **5** and **11** were combined with two commercial antibiotics kanamycin and chloramphenicol, they showed strong synergistic activity at sub-MIC levels against *S. aureus* USA300 and *B. subtilis* 168. Moreover, chloramphenicol turned from a bacteriostatic to a bactericidal agent when combined with compound **11** against *B.subtilis* 168.

Key words: triterpenoid saponin；ocotillol-type；antibacterial activity；synergistic effect

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/cbdd.12337

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

Introduction

The process of discovery, development and marketing of antibacterials used to be dynamic. However, this phenomenon changed in late 20th century, when the commercialization of new antibacterials slowed down even though most new drug development focused on modification of existing antibiotics. A major problem has been the emergence of 'superbugs' where bacteria have developed resistance to some or all clinically useful antibiotics (1). This resistance developed over short time periods, and as a result rendered the R&D of new antibiotics commercially challenging for the pharmaceutical industry (2). This has created an urgent need for the discovery of new antibacterial agents along with maximizing the utility of old or clinically discarded antibiotics.

Plant derived triterpenoid saponins have been shown to be involved in plant defense systems by targeting the membrane of bacteria and enveloped viruses (3,4), as well as increasing the membrane permeability of mammalian cells (5). Triterpenoids increase cell membrane permeability by forming pore-like channels (6), and a very recent report demonstrated that the saponin (α-hederin) sugar chains are required to efficiently interact with sterols in the membrane for pore formation and budding. The triterpenoid aglycone, without sugar moieties, only provided poor intravesicular budding (Figure 1, α-hederin) (7). Cationic triterpenoids have also been described in the literature that show a detergent-like antibacterial effect which is generated by their amine-enriched structures (Figure 1, CSA) (8). A classic triterpenoid antibiotic, fusidic acid, is clinically used to treat Gram positive bacterial infection. However, studies showed that as a protein synthesis inhibitor, this drug targeted ribosomal translocase enzyme rather than cell membrane (9).

Figure 1. Structures of α-hederin, CSA, fusidic acid and compound **3**

We previously reported novel ocotillol-type triterpenoid derivatives without sugar chains or amines that still possessed good antibacterial activity (10). Furthermore, these hydrophilic compounds displayed significant synergistic effects against several bacterial species. This impressive result encouraged us to explore the relationship between the structure and the bioactivity of these compounds.

In our previous study (10), we demonstrated that triol **3** displayed good antibacterial activity against *S.aureus* and *B. Subtilis* with minimum inhibitory concentrations (MIC) of 8 μg/mL against both organisms (Figure 1, compound **3**). When a sub-MIC of triol **3** was combined with kanamycin (KAN), the latter displayed enhanced antibacterial activity with MIC values of 0.125 μg/mL against *S.aureus* (1 μg/mL when KAN was used alone). The phthalic mono ester substitution at 12-OH furnished similar antibacterial activities but with a clearly improved synergistic effect when combined with chloramphenicol (CHL) or kanamycin (KAN: 0.0078 μg/mL vs 1 μg/mL and CHL: 0.016 μg/mL vs 4 μg/mL against *S. aureus;* KAN: <0.0020 μg/mL vs 0.25 μg/mL and CHL: <0.0078 μg/mL vs 2 μg/mL against *B. subtilis*). However, modifications of the 3-OH and 12-OH groups to ketones dramatically diminished the activity of the ocotillol-type triterpenoid derivatives.

As shown in Figure 2, the electrostatic potential surface (wire mesh) of the energy minimized geometry of triol **3** was calculated using Accelrys DS 2.5 (11), the critical 3-OH on the left-hand side of hydrophobic triterpenoid core is relatively far from other hydrogen-donor or accepter atoms. As a result, the 3-OH should be suitable for modification to improve the activity of this compound without affecting other potential binding sites. In this paper, aromatic substitutions on 3-OH were performed and their antibacterial activity evaluated against several representative bacteria.

Figure 2. Electrostatic potential surface (wire mesh) of energy minimized geometry of triol **3**.

Methods and Materials

Most chemicals and solvents were analytical grade and, when necessary, were purified and dried with standard methods. Melting points were taken on an XT-4 micro melting point apparatus and uncorrected. ^{1}H NMR spectra were recorded with a Bruker AV-300 or ACF 500 spectrometer in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in δ values (ppm) and the coupling constants (*J*) in Hz. High-resolution mass spectra were recorded using an Agilent QTOF 6520. The antibacterial and synergistic antibacterial activity were examined as described (10, 11)

Triols 3 and 4 were synthesized as described (10). Typical procedure for the synthesis of **5**-**16**: To a solution of **3** or **4** (70 mg, 0.15 mmol) and DMAP (25 mg, 0.18 mmol) in dry dichloromethane (4 mL) was added an acid or an anhydride (0.30 mmol). The reaction mixture was stirred at room temperature for 5 h, then the solution was washed with saturated aqueous $NaHCO_3$ solution, water and brine, then dried over anhydrous sodium sulfate and concentrated. The organic mixture was purified by silica gel column chromatography (petroleum ether: ethyl acetate=6:1) to provide the desired product.

Results and Discussion

Chemistry

The synthesis was carried out with **PPD**, one of the main components of *Panax ginseng*, as the starting material (12). Protection of the 3,12-diol groups of **PPD** as a diacetate in the presence of DMAP and acetic anhydride resulted in compound **1**, followed by epoxidation and cyclization *in situ* to form the E ring of compound **2** as a mixture of epimers. After base treatment, triols **3** and **4** were obtained with excellent yields.

To understand the structure and activity relationship on the 3-OH of two diastereomers, triol **3** was then allowed to react with phthalic anhydride, niacin, cinnamic acid, benzoic acid, 2-furoic acid and salicylic acid to provide the corresponding derivatives **5**-**10**.The same procedure applied to triol **4** formed compounds **11**-**16,** respectively.

Scheme 1. The synthesis of ocotillol-type derivatives 5-16. Reagents and conditions: i, Ac_2O, DMAP; ii, mCPBA; iii, KOH; iv, anhydride or acids, EDCI, DMAP.

The absorption, distribution, metabolism and excretion (ADME) properties of all the compounds were calculated using Accelrys DS 2.5. As shown in table 1 column 2, most of the derivatives demonstrated similar *in vivo* solubility, except compounds **13**-**15** bearing apolar aromatic groups. The calculated logarithm of the partition coefficient (cLogP) displayed similar results. However, the uncorrelated cLogP and cLogD values (column 3 and 4) of **5** and **11** showed that these two compounds are ionizable, which correspond to their higher 2D polar surface area (Column 5) to possess better passive transport ability through cell membranes than other compounds. While lower value for absorption level indicates better absorption (Column 6), thus compounds **5**-**10** with 24*S* stereochemistry showed slightly improved absorption than their epimers and the starting materials.

Table 1. ADME property prediction of compound PPD and **3**-**16**

All of the compounds displayed similar properties at the blood brain barrier level (Table 1, Column 4) and the plasma protein binding level (Table 1, Column 5), which indicate they barely pass the blood brain barrier, however have very high plasma protein binding ability. Very low hepatotoxicity prediction of all the compounds

demonstrates that their metabolism in *vivo* should be safe, which correlates to our previous study against human cell lines (10).

Antibacterial activity

The antibacterial activity of compounds **5**-**16** was evaluated against several representative Gram-positive and Gram-negative bacteria species: *Bacillus subtilis* 168 (13), *Staphylococcus aureus* RN4220, *Escherichia coli* DH5α, *Acinetobacter baumannii* 19606 and *Pseudomonas aeruginosa* PAO1. Initial minimum inhibitory concentration (MIC) results are shown in Table 2. The data demonstrated that compounds **PPD**, **3**, **5** and **11** displayed good antibacterial activity against Gram-positive bacteria with MIC values of 1-32 μg/mL, in which phthalic mono esters **5** and **11** exhibited excellent antibacterial activity with MICs of 1 μg/mL against *S. aureus* RN4220, respectively. Compared to triol **3**, the inhibitory effects of compound **5** were significantly improved. Furthermore, the dramatic difference in antibacterial activity between **3** and **4** caused by stereochemistry on the 24-isopropanol seemed to be overpowered by substitutions at 3-OH (10). Another evident feature was the antibacterial activity of compound **11** against *B. Subtilis* 168, the MIC of 4 μg/mL was comparable to that of **5** which had an MIC of 8 μg/mL. This surprising result confirmed the significance of substitutions at 3-OH.

Table 2. MIC values (μg/ml) against Gram-(+) and Gram-(-) bacteria

Nicotinates **6** and **12** only showed mild activity against *S. aureus* RN4220 with MICs of 64 and 128 μg/mL; Cinnamate **13** could mildly inhibit the growth of *S. Aureus* while its (*S*)-diastereomer **7** only displayed activity against the Gram-negative bacterium *P. aeruginosa* PAO1 with an MIC value of 64 μg/mL. Benzoate **8** didn't show activity against any bacteria, and its epimer **14** only inhibited *S. Aureus* RN4220 growth. Their activities are probably due to the relatively a polar aromatic substitution compared to other compounds.

Furan carboxylate **9** was effective against *S. aureus* at 128 μg/mL, however, its (*R*)-diastereomer **15** demonstrated no activity against Gram positive bacteria. Unlike hydroxybenzoate **10**, its (*S*)-diastereomer **16** showed activity against *S. aureus* RN4220 with an MIC of 64 μg/mL.

With similar structural substitution, compounds **5**-**16** displayed enormous differences in antibacterial activity against Gram positive bacteria. **5** and **11** bearing a phthalic acid at 3-carboxylate provided enhanced activity compared to **3** and **4** with a hydroxyl group, whereas structurally similar compounds **10** and **16** carrying an *o*-phenol only had mild activity, similar to non-hydrogen donors **6**-**9** and **12**-**15**. Given the pKa difference between phthalic acid and phenol (3.0 vs 9.7), the acidity of 3-substitution may play an important role in antibacterial activity. With regard to Gram negative bacteria, phthalic mono ester **5** displayed mild activity against *E. coli*, *P. aeruginosa* and *A. baumannii*; furan carboxylate **9**, compound **11**, nicotinate **12** and cinnamates **7**, **13** could moderately inhibit the growth of *P. aeruginosa*, while cinnamate **13** could also act against *E. coli*. Additionally, compounds **10**, **15** and **16** showed activity against *A. baumannii* at 128 μg/mL. The above results revealed aromatic substitutions on 3-OH have less influence on the activity of compounds against Gram negative bacteria. It is worth noting that our previous study had shown that some compounds with substitution at the 12-OH possess moderate activity against Gram negative bacteria (10), suggesting that further improvement of activity against Gram negative bacteria may be dependent on the appropriate chemical modification of that group.

As shown in Table 3, the bioactive compounds against Gram-(+) bacteria were chosen for further testing against a significant highly pathogenic community-acquired methicillin resistant strain of *S. Aureus* (CA-MRSA) USA300. Phthalic mono esters **5** and **11** displayed good antibacterial activity against *S. aureus* USA300 with MICs of 4 μg/mL, respectively, while nicotinates **6** and **12**, as well as cinnamates **7** and **13** retained their mild activity against this pathogen.

Table 3. Antibacterial activity of compounds **5**-**7** and **11**-**13** against MRSA[a] (MIC: μg/mL)

Compounds **5** and **11** showed greater antibacterial activity when compared against the compounds we previously reported (10). As a result, their minimum bactericidal concentrations (MBCs) were further investigated. Phthalic mono esters **5** and **11** exhibited similar bactericidal ability (**5**: 32 μg/mL against *S. aureus* RN4220 and *S. aureus* USA300, 16 μg/mL against *B. subtilis* 168; **11**: 32 μg/mL against *S. aureus* RN4220, *S. aureus* USA300 and *B. subtilis* 168) which was a significant improvement on compounds **3** and **4** with hydroxyl at 3-position (10).

Figure 3. Molecular overlapping of compound **3** (green) and **4** (purple) (top), compound **3** (green) and **11** (gray) (bottom).

These results encouraged us to conduct structure comparison by using Accelrys DS 2.5 (11). After energy minimization, three molecules **3**, **4** and **11** were superimposed and overlayed. As shown in Figure 3, (*R*)-triol **4** (purple) has a distorted C-24 over its (*S*)-epimer **3** (green) and with the isopropanol group rotated (Figure 3, top arrow). Taking into account the difference in melting points (**3**, mp. 224-225℃; **4**, mp. 167-169℃) and the different Rf values (**3**, Rf ≈ 0.2; **4**, Rf ≈ 0.4, solvents: petroleum ether / ethyl acetate = 2/3) in thin layer chromatography, we assumed that triol **3** tends to form an intermolecular hydrogen bonding interaction between the 12-OH and tetrahydrofuran ring, while the (*R*)-epimer **4** is more apt to be involved in intramolecular hydrogen bonding (14,15). This hydrogen bonding interaction may affect the accessibility of hydrogen bond donors/acceptors to the 12-OH and E-ring of the epimers. In contrast, the phthalic mono ester substitution seemed to affect the structure of (*R*)-ester **11** (gray), which causes the tetrahydrofuran ring to stay at a lower position to fit onto the E-ring of triol **3** and bypasses this steric inequality (Figure 3, bottom arrow). This structural and physicochemical characterization could account for the difference in antibacterial activity between this pair of diastereomers. A plausible mechanism of action may be for the triterpenoid skeleton to provide a hydrophobic surface while the hydroxyl/E-ring and the carboxylic acid function as a polar head and tail to form a polar-apolar-polar amphiphile (16). This amphiphilic topology with appropriate physicochemical properties could play an important role by causing membrane damage or facilitating the uptake of the triterpenoids and antibiotics into cells (17).

Synergistic antibacterial activity

Antibacterial agents targeting unrelated bacterial functions or processes may synergistically enhance their bioactivity when used in combination, and the clinical application of combinatorial therapy is very common in the treatment antibiotic resistant bacterial infections (18). As plant derived triterpenoids target the cell membrane, these compounds may be suitable for synergistic combination with antibiotics that target intracellular processes. We have recently reported that ocotillol-type triterpenoid derivatives demonstrated synergistic effects against *Bacillus subtilis* 168 and *Staphylococcus aureus* USA300 in combination with bacterial protein synthesis inhibitors kanamycin and chloramphenico (10). Therefore, the two phthalic mono ester epimers **5** and **11** were also chosen to study the impact of the substitution at 3-OH on synergistic antibacterial activity.

Table 4. Synergistic effect of different antibiotics with compounds 5 and 11 against S. aureus USA 300 and B. subtilis 168

As shown in Table 4, compounds **5** and **11** reduced the MICs of kanamycin against *S. aureus* USA300 from 1 μg/mL to 0.0625 and 0.0156 μg/mL, respectively (FICI=0.078, 0.020). Synergistic inhibition against *B. subtilis* 168 was also observed when **5** was combined with kanamycin or chloramphenicol (FICI = 0.13, 0.156). Furthermore, the MICs of kanamycin and chloramphenicol were dramatically reduced against *B. Subtilis*168 from 0.25 and 2 μg/mL to 0.0039 and 0.0020 μg/mL in combination with **11** with FICI values of less than 0.017 and 0.0015, respectively. An additive effect (FICI =1) was observed when **5** or **11** was combined with chloramphenicol against *S. aureus* USA300. A study showed that fusidic acid didn't display any synergistic effect when combined with chloramphenicol (19), which illustrated our compounds may target elsewhere rather than bacterial ribosome as fudisic acid does, despite of similar chemical skeletons.

To enter the bacterial cytoplasm, lipid-mediated transportation of kanamycin helps the drug to cross bacterial membrane (20), while chloramphenicol can be transported by both lipid and porin pathways (21). As indicated in Table 4, compounds **5** and **11** can synergistically enhance the antibacterial activity of kanamycin and chloramphenicol against Gram positive bacteria. If only porin transportation was affected, compounds **5** and **11** would not improve the activity of kanamycin. The result suggested compounds **5** and **11** may target the phospholipid-mediated pathway. Additionally, compounds **5** and **11** didn't demonstrate activity or synergistic effect against Gram negative bacteria, which maintain an unique outer membrane constructed by lipopolysaccharides (LPS). The lipopolysaccharides consisting lipid and polysaccharides may be the cause for ineffectiveness of our compounds against Gram negative bacteria, while α-hederin containing a sugar side chain can reasonably penetrate into the LPS of *E. coli* (7).

Additionally, the bactericidal activities of the above two compounds in combination with the antibiotics were also examined. When combined with compound **5** and/or **11**, the MBCs of kanamycin against *B. subtilis* 168 were significantly reduced from 1 μg/mL to 0.25 and 0.0312 μg/mL, respectively. While for *S. aureus* USA300, improved bactericidal activity was also observed when compound **11** combined with kanamycin with an MBC decrease from 4μg/mL to 0.25 μg/mL. Surprisingly, when using chloramphenicol alone it was bacteriostatic against *B. subtilis* 168, but it displayed good bactericidal activity when combined with **11** with an MBC value of 1 μg/mL. The result illustrated that at that concentration, the amount of intracellular chloramphenicol was sufficient to suppress bacterial protein synthesis and then cause cell death before the antibiotic resistance occurs.

As a result, compounds **5** and **11** with phthalic mono acid substituted at the 3-OH both retained the synergistic activity of their homologous triols **3** and **4**. The ameliorated antibacterial ability and retention of synergistic ability demonstrated that the acidic substitution at 3-OH provided improved performance of these triterpenoid derivatives as antibacterials.

Based on the data above and our recently reported findings (10), a more comprehensive structure-activity relationship could be developed and is shown in Figure 4: hydrogen bond donors at C-3 and C-12 are required for activity against Gram positive bacteria; the functional groups at C-3 and C-12 act as hydrogen bond acceptors when converted to ketones and show decreased activity; a non-hydrogen bond donor ester substitution at C-12 showed mild activity against Gram negative bacteria; the (24*S*)-configuration is preferred for antibacterial activity of compounds without substitution at the 3-OH; substitution at 3-OH can cause changes in molecular conformation resulting in bioactive (24*R*)-compounds; at the 3-OH, polar aromatic substitutions furnish activity against both Gram positive and negative bacteria, while acidic substitution is preferred and efficiently enhances activity against Gram positive bacteria, including community-acquired methicillin resistant *S. aureus* USA300.

Figure 4. Preliminary SARs of ocotillol-type derivatives

Conclusions

In summary, through a straight forward synthetic route, a series of novel ocotillol-type triterpenoid derivatives have been efficiently synthesized that display good antibacterial activity (~ 1 μg/mL) and synergism when combined with kanamycin and chloramphenicol. These compounds are nitrogen and sugar free, appear to represent a new class of antibacterial triterpenoid, and similar compounds demonstrate low cytotoxicity against several human cell lines (10). Additionally, old drugs like kanamycin and chloramphenicol could again serve as useful antibacterials with reduced dosage and thus lowered toxicity when used together with these ocotillol-type triterpenoid derivatives. Our findings provide further insight into the biological effects of the triterpenoids that may be useful in the future development of natural plant derived antibacterial products. Detailed structure-activity relationship with mechanistic study of these ocotillol-type triterpenoid derivatives will be carried out in the near future in our laboratories.

Acknowledgements

The authors are grateful to National Natural Science foundation of China (No. 81001358 and 51272223), Promotive research fund for excellent young and middle-aged scientists of Shandong Province (No. BS2010YY073), Shandong Provincial Natural Science Foundation, China (No. ZR2012HM036), Open Project Program of State Key Laboratory of Natural Medicines, China Pharmaceutical University (No.201201) and Project for Research and Innovation of Graduates in Universities of Jiangsu Province (CXLX13_306) for financial assistance, Taishan Scholar Project to Fenghua Fu and Australian NHMRC grant APP1008014 for financial support.

Conflict of Interest

The authors declare no competing financial interest.

REFERENCES

1 Arias, C. A.; Murray, B. E. (2009) Antibiotic-Resistant Bugs in the 21st Century — A Clinical Super-Challenge. *N. Engl. J. Med*; *360*: 439-443.
2 Laidman, J. (2013) Despite Growing Crisis, Few New Antibiotics Are in Pipeline. *Medscape Medical News*; Apr 22.
3 Hill, R. A.; Connolly, J. D. Triterpenoids. (2012) *Nat. Prod. Rep*; *29*: 780-818.
4 Augustin, J. M.; Kuzina, V.; Andersen, S. B.; Bak, S. (2011) Molecular activities, biosynthesis and evolution of triterpenoid saponins. *Phytochemistry*; *72*: 435-457.
5 Melzig, M. F.; Bader, G.; Loose, R. (2001) Investigations of the mechanism of membrane activity of selected triterpenoidsaponins. *Planta Med*; *67*: 43-48.
6 Li, X. X.; Davis, B.; Haridas, V.; Gutterman, J. U.; Colombini, M. (2005) Proapoptotic Triterpene Electrophiles (Avicins) Form Channels in Membranes: Cholesterol Dependence. *Biophys J*; *88*: 2577-2584.
7 Lorent, J., Le Duff, C. S., Quetin-Leclercq, J., & Mingeot-Leclercq, M. P. (2013) Induction of Highly Curved Structures in Relation to Membrane Permeabilization and Budding by the Triterpenoid Saponins, α-and δ-Hederin. *Journal of Biological Chemistry*; *88*: 14000-14017.

8 Ding, B.; Guan, Q.; Walsh, J. P.; Boswell, J. S.; Winter, T. W.; Winter, E. S.; Boyd, S. S.; Li, C.; Savage, P. B. (2002) Correlation of the Antibacterial Activities of Cationic Peptide Antibiotics and Cationic Steroid Antibiotics. *J. Med. Chem*; *45*: 663-669.
9 Tanaka, N.; Kinoshita, T.; Masukawa, H. (1969) Mechanism of inhibition of protein synthesis by fusidic acid and related steroidal antibiotics. *J. Biochem*; *65*: 459-464.

10 Zhou, Z.; Ma, C.; Zhang, H.; Bi, Y.; Chen, X.; Tian, H.; Xie, X.; Meng, Q.; Lewis, P. J.; Xu, J. (2013) Synthesis and Biological Evaluation of Novel Ocotillol-Type Triterpenoid Derivatives as Antibacterial Agents. *Eur. J. Med. Chem*; *68*: 444-453.
11 Ma, C.; Yang, X.; Kandemir, H.; Mielczarek, M.; Johnston, E. B.; Griffith, R.; Kumar, N.; Lewis, P. J. (2013) Inhibitors of Bacterial Transcription Initiation Complex Formation. *ACS Chem. Biol*: *8*: 972-1980.
12 Seiji, F.; Ryoji, K.; Kazuhiro, O.; Kazuo, Y.; Chiu, M. H.; Nie, R. L.; Osamu, T. (1995) Dammarane glycosides from aerial part of *Neoalsomitra Integrifoliola*. *Phytochemistry*;*39*:591-602.
13 *B. Subtilis* was used as an initial model representative of the spore-forming firmicutes that includes pathogens such as *B. cereus* and *B. anthracis*.
14 Gur'yanova, E. N.; Gol'dshtein, I. P.; Perepelkova, T. I. (1976) The Polarity and Strength of the Intermolecular Hydrogen Bond. *Russ. Chem. Rev*; *45*: 792-806.
15Simperler, A.; Watt, S. W.; Bonnet, P. A.; Jones, W.; Motherwell, W. D. S. (2006) Correlation of melting points of inositols with hydrogen bonding patterns. *Cryst Eng Comm***;** *8*: 589-600.
16 Böhme, S.; Padmavathi, P. V. L.; Holterhues, J.; Ouchni, F.; Klarea, J. P.; Steinhoff, H. J. (2009) Topology of the amphipathic helices of the colicin A pore-forming domain in *E. Coli* lipid membranes studied by pulse EPR. *Phys. Chem. Chem. Phys*; *11*: 6770-6777.
17 Findlay, B.; Zhanel, G. G.; Schweizer, F. (2010) Cationic Amphiphiles, a New Generation of Antimicrobials Inspired by the Natural Antimicrobial Peptide Scaffold. *Antimicrob. Agents Chemother*; *54*: 4049-4058.
18 Vincent, W. R.; Martin, C. A.; Winstead, P. S.; Smith, K. M.;Gatz, J.; Lewis, D. A. (2009) Effects of a Pharmacist-to-Dose Computerized Request on Promptness of Antimicrobial Therapy. *J. Am. Med. Inform. Assoc*; *16*: 47–53.
19 Wright, G. L.; Harper, J. (1970) Fusidic acid and lincomycin therapy in staphylococcal infections in cystic fibrosis. *Lancet*; *7636*: 9-14.

20 Delcour, A. H. (2009) Outer Membrane Permeability and Antibiotic Resistance. *Biochim. Biophys. Acta***;** *1794*: 808-816.

21 Mortimer, P. G.; Piddock, L. J. (1993) The accumulation of five antibacterial agents in porin-deficient mutants of *Escherichia coli*. *J. Antimicrob. Chemother*; *32*: 195-213.

22 Chan, B.C.L.;Ip, M.; Gong, H.;Lui, S.L.; See, R.H. (2013) Synergistic effects of diosmetin with erythromycin against ABC transporter over-expressed methicillin-resistant *Staphylococcus aureus* (MRSA) RN4220/UL5054 and inhibition of MRSA pyruvate kinase. *Phytomedicine*; *20*: 611-614.

Supporting Information

Additional Supporting Information may be found in theonline version of this article:

SI. ^{1}H NMR, ^{13}C NMR, HRMS and IR data for all compounds synthesized.

Table 1. ADME property prediction of compound PPD and **3**-**16**

	Solubility	cLogP	cLogD	PSA 2D	Absorption Level	BBB Level	PPB Level	Hepato-toxicity
PPD	-6.923	7.124	7.124	62.446	3	4	2	0
3	-6.566	5.822	5.822	71.376	3	4	2	0
4	-6.566	5.822	5.822	71.376	3	4	2	0
5	-6.071	6.08	4.687	118.422	2	4	2	0
6	-6.25	5.38	5.38	88.053	1	4	2	0
7	-7.018	6.999	6.999	76.791	2	4	2	0
8	-7.084	6.531	6.531	76.791	2	4	2	0
9	-6.852	5.926	5.926	89.346	2	4	2	0
10	-6.701	6.289	6.288	97.607	2	4	2	0
11	-7.719	7.495	6.022	114.908	3	4	2	0
12	-7.409	6.715	6.715	88.053	2	4	2	0
13	-8.177	8.333	8.333	76.791	3	4	2	0
14	-8.244	7.866	7.866	76.791	3	4	2	0
15	-8.011	7.261	7.261	89.246	3	4	2	0

16	-7.86	7.624	7.622	97.607	3	4	2	0

cLogP: calculated logarithm of the partition coefficient; cLogD: calculated logarithm of the distribution constant; PSA 2D: 2D polar surface area; BBB: blood brain barrier; PPB: plasma protein binding.

Table 2. MIC values (μg/ml) against Gram-(+) and Gram-(-) bacteria

mg/L	*S.aureus*	*B.sub*	*E.coli*	*P.aer*	*A.baum*
PPD	16	32	64	>128	64
3	8	8	>128	>128	>128
4	64	128	>128	>128	128
5	1	8	64	128	128
6	64	128	>128	>128	>128
7	128	>128	>128	64	>128
8	>128	>128	>128	>128	>128
9	128	>128	>128	128	>128
10	>128	>128	>128	>128	128
11	1	4	>128	128	>128
12	128	>128	>128	128	>128
13	64	>128	128	128	>128
14	64	>128	>128	>128	>128
15	>128	>128	>128	>128	128
16	64	>128	>128	>128	128
KAN	2	0.5	1	8	1

Table 3. Antibacterial activity of compounds **5**-**7** and **11**-**13** against MRSA[a] (MIC: µg/mL)

Compound	*S. aureus* USA300
PPD	32
3	8
5	4
6	64
7	128
11	4
12	128
13	64
KAN[b]	1

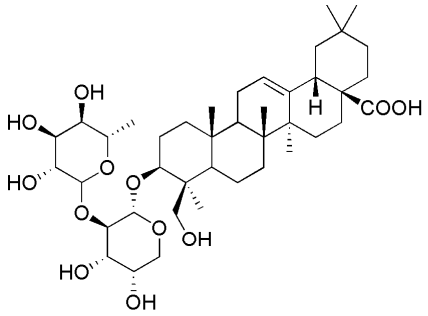
[a]MRSA: methicillin-resistant *S. aureus* USA300

[b]KAN: kanamycin

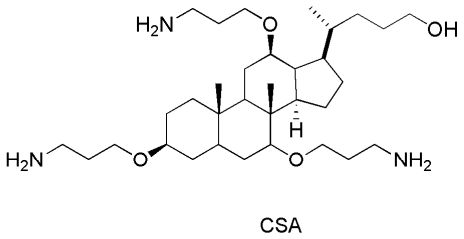
Table 4. Synergistic effect of different antibiotics with compounds **5** and **11** against *S. aureus* USA 300 and *B. subtilis* 168

Compound	MIC (μg/mL)		MBC (μg/mL)		FICI (FIC index)[d]	
	S. aureus USA300	*B.sub* 168	*S. aureus* USA300	*B.sub* 168	*S. aureus* USA300	*B.sub* 168
KAN[a]	1	0.25	4	1	-	-
CHL[b]	4	2	N/A[c]	N/A	-	-
5+KAN	0.0625	0.0312	4	0.25	0.078	0.13
11+KAN	0.0156	<0.0039	0.25	0.0312	0.020	<0.017
5+CHL	2	0.25	N/A	N/A	1	0.156
11+CHL	2	<0.0020	N/A	1	1	<0.0015

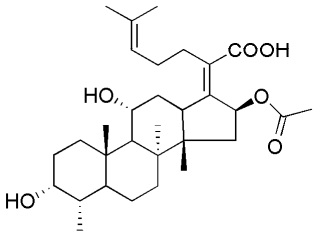
[a]KAN: kanamycin; [b]CHL: chloramphenicol; [c]N/A: not applicable. [d]FICI: according to the literature(22): FIC of drug A (FIC A) = MIC of drug A in combination/MIC of drug A alone; FIC of drug B (FIC B) = MIC of drug B in combination/MIC of drug B alone; hence FICI = FIC A + FIC B. "Synergy" was defined when FICI was less than or equal to 0.5; while "additive" in which the FICI was greater than 0.5 and less than or equal to 1.0; whereas "indifferent" when the FICI was greater than 1.0 and less than or equal to 2.0; and "antagonistic" in cases which the FICI was greater than 2.0.



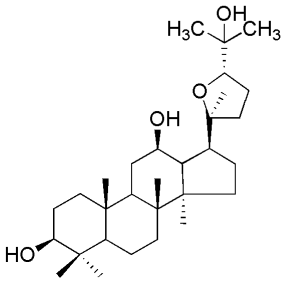
a-hederin



CSA



Fusidic acid



3